Random Amplified Polymorphic DNA Reveals Relationships Among Diverse Genotypes in Australian and American Collections of *Uromyces appendiculatus*

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This work was supported by a grant from the Australian Research Council to the first and third authors.

We thank K. Kazan for advice with random amplified polymorphic DNA methods, and B. Engel for useful comments on the manuscript. We thank R. Redden, R. O'Brien, L. Tesoriero, A. Akehurst, and the late K. Middleton for assistance with the collection of Australian isolates of bean rust. Thanks also to P. Martinez for extracting DNA from the five American isolates. We are grateful to B. Tyler for helpful discussions and statistical advice.

Accepted for publication 23 March 1995.

ABSTRACT

Maclean, D. J., Braithwaite, K. S., Irwin, J. A. G., Manners, J. M., and Groth, J. V. 1995. Random amplified polymorphic DNA reveals relationships among diverse genotypes in Australian and American collections of *Uromyces appendiculatus*. Phytopathology 85:757-765.

Random amplified polymorphic DNA (RAPD) markers were used to assess genetic diversity among 42 Australian and five American field isolates of the rust fungus *Uromyces appendiculatus* var. *appendiculatus*, pathogenic on cultivated bean *Phaseolus vulgaris*. Phenetic analysis of polymorphisms generated by 10 arbitrary decanucleotide primers indicated that the Australian isolates represented two background genotypes, A and B (17% band dissimilarity), with a third group (genotype AB) representing putative hybrids and recombinants between A and B. This confirms a previous restriction fragment length polymorphism and RAPD study carried out on a subset of 12 Australian isolates. The American isolates, of unknown homology to the Australian isolates prior to this investigation, were selected to represent the two major genetic groups determined previously using isozymic markers. RAPDs confirmed the isozymic groupings and revealed that one group, represented by one American isolate, clustered with the Australian isolates of genotype B. However, the other four American isolates formed a divergent cluster, designated genotype C, that showed an average band dissimilarity of 45% compared with genotypes A and B. A graphic display of polymorphic RAPD bands was used to compare genomic components common to different genotype clusters. This revealed a group of RAPD markers common to genotypes C and A but absent from B. The data were consistent with A being derived by recombination between progenitors of genotypes B and C. Although genotype C is commonly found in North America, its absence from the 42 Australian isolates suggests that it is either uncommon or has not yet been introduced to Australia. Results are discussed in relation to the evolution and possible mechanisms of generation of current genotypes of *U. appendiculatus*.

Additional keywords: bean rust, polymerase chain reaction.

The rust fungus *Uromyces appendiculatus* (Pers.:Pers.) Unger var. *appendiculatus*, a pathogen of common bean *Phaseolus vulgaris* L., has a worldwide distribution and causes major production problems in humid tropical and subtropical regions and occasionally in temperate regions (24). The fungus is one of the most variable plant pathogens with respect to race phenotype, with over 200 races identified worldwide including over 70 in North America (24,25) and over 25 in Australia (1). Bean rust is believed to have coevolved with its hosts in Central and South America, and has presumably spread to other regions with the cultivation of common bean or other related host species (24). Because of the geographic isolation of Australia from the center of origin in the Americas, it was predicted prior to this investigation that the Australian population of bean rust may represent a subset of the background genotypes present in the Americas.

Genetic differences between strains of host-specific fungal phytopathogens can be assessed by analysis of race phenotype and background genotype. The race phenotype of plant pathogenic fungi is controlled by relatively few loci (9,20), and may give no indication of the extent of other genetic differences either between races or between independent collections of a race. However, the extent of overall genetic differences can be assessed by comparing the "background genotype" of isolates at loci arbitrarily selected across the genome.

The relationship between race phenotype and background genotype in *U. appendiculatus* was first examined by Linde et al. (14), who used isozyme markers to assess differences in background genotype among 23 geographically diverse North and Central American isolates, three laboratory hybrids, and one European isolate. Among 55 enzyme and protein systems tested, polymorphisms were detected in 13 isozyme systems representing 15 loci. Phenetic analysis of these polymorphisms revealed three genetically diverse clusters, one of which consisted of a laboratory hybrid and a naturally occurring isolate. Although analysis of these isolates on 18 differential bean lines revealed greater diversity for virulence than for isozyme markers, phenograms based on virulence differences grouped the isolates similarly to phenograms based on isozyme differences (14). These data showed that the race phenotype of American isolates correlated with isozyme group, and implied a relationship between virulence and isozyme groupings. The one European isolate included in the above study fitted within the predominant American group of isolates (14); no Australian isolates were included in the study.

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TABLE 1. Isolates of *Uromyces appendiculatus* included in this study

Isolate number[a]	Accession code[b]	Date collected	Location and notes	Background genotype
i) Old collections (1970s)				
1	UA19	1974	Rydalmere, NSW, (A)[c]	A
2	UA21	1975	Clare, Qld, (A)[c]	A
3	UA22	1974	Valla, NSW, (A)[c]	A
4	UA23	1974	Rydalmere, NSW[c]	A
5	UA25	1974	Rydalmere, NSW[c]	A
6	UA32	1974	Rydalmere, NSW, (A)[c]	A
7	UA24	1975	Rydalmere, NSW, (A)[c]	A[d]
8	UA26	1974	Copmanhurst, NSW	A[d]
9	UA35	1976	Lindenow, Vic[c]	A[d]
10	UA34	1974	Unknown, (AB)[c]	AB
11	UA33	1974	Unknown[c]	A
12	UA31	1977	Unknown[c]	A
ii) Recent collections (1989 or later): Brisbane region				
13	UA1	1989	Rocklea, Qld[e]	AB
14	UA2	1989	Rocklea, Qld[e]	B
15	UA3	1989	Rocklea, Qld[e]	A
16	UA4	1989	Rocklea, Qld[e]	A
17	UA7	1989	Rocklea, Qld[e]	A
18	UA10	1989	Rocklea, Qld[e]	A
19	UA11	1989	Rocklea, Qld[e]	A
20	UA30	1990	St. Lucia, Qld	A[d]
iii) Qld coastal region near north of Brisbane				
21	UA27	1990	Gympie, Qld, (B)[c]	B
22	UA28	1990	Gympie, Qld, (B)[c]	B
23	UA29	1990	Gympie, Qld[c]	B[f]
24	UA54	1991	Cooroy, Qld	AB
25	UA53	1991	Unknown[c]	B
iv) Inland Qld west of Brisbane				
26	UA37	1990	Gatton, Qld[c]	B[f]
27	UA39	1990	Kingaroy, Qld[e]	AB
28	UA40	1990	Kingaroy, Qld[e]	AB
29	UA41	1990	Kingaroy, Qld[e]	B[f]
30	UA42	1990	Kingaroy, Qld[e]	B[f]
31	UA43	1990	Kingaroy, Qld[e]	B
32	UA44	1990	Kingaroy, Qld[e]	AB
33	UA45	1990	Kingaroy, Qld (B)[c,e]	B
34	UA46	1990	Kingaroy, Qld (B)[c,e]	B
35	UA47	1990	Kingaroy, Qld[c,e]	B
36	UA48	1990	Kingaroy, Qld[c,e]	B
37	UA49	1990	Kingaroy, Qld (AB)[c,e]	AB
38	UA50	1990	Kingaroy, Qld[e]	AB
39	UA51	1990	Kingaroy, Qld[e]	AB
v) NSW mid to north coastal regions				
40	UA64	1992	Pennant Hills, NSW	A[d]
41	UA65	1992	Riverstone, NSW	AB
42	UA66	1992	Macksville, NSW	AB
A1[g]	W73-2	1973	Wisconsin (nontelial)	B
A2[g]	P10-1	1976	North Dakota (produces telia)	C
A3[g]	S1-5	1977	Michigan (produces telia)	C
A4[g]	S3-1	1977	Minnesota (nontelial)	C
A5[g]	Drep-1	NT[h]	Dominican Republic (few telia)	C

[a] Isolates 1 through 42 are Australian; A1 through A5 are American.

[b] University of Queensland collection number (Australian isolates), or letter code described previously (14,15) for the American isolates.

[c] Isolates included in previous study (3). Parentheses indicate background genotype of those isolates in previous study analyzed by full set of 10 random amplified polymorphic DNA (RAPD) primers and 10 restriction fragment length polymorphism probes (3).

[d] These isolates also exhibited one of the RAPD bands characteristic of genotype B (cf. Fig. 1).

[e] Isolates 13 to 19 and 27 to 39 represent sets of isolates collected from different bean lines grown in the same field as part of a "bean rust nursery" composed of a set of bean cultivars (24).

[f] These isolates also exhibited one or two RAPD bands characteristic of genotype A (cf. Fig. 1).

[g] These American isolates selected to represent diverse members of the isozyme groups detected previously (14).

[h] Not known.

More recently, Braithwaite et al. (3) used DNA markers to compare the background genotypes of 22 isolates of bean rust collected from diverse locations in Australia spanning the period 1973 to 1990. Two classes of marker, restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPDs), independently revealed the existence of three clusters of isolates. Detailed analysis of a subset of 12 isolates revealed that two of the clusters, designated A and B, represented genetically diverse background genotypes, while isolates within the third cluster (designated AB) exhibited most of the polymorphic DNA markers given by both A and B. No polymorphic bands (RFLPs or RAPDs) were unique to the AB cluster, indicating that isolates within this cluster had not evolved independently for sufficient time to observe unique genomic sequences. It was concluded that isolates with an AB genotype had most probably arisen from recent hybridization between isolates of background genotypes A and B (3,16). Analysis on host differentials revealed 10 race phenotypes among the subset of 12 isolates, but unlike the study of American isolates (14) there was no evidence of a correlation between race phenotype and background genotype.

Different molecular markers were used to assess background genotype in the studies of American (14) and Australian (3) collections of bean rust summarized above. Thus, the above studies provided no information on the genetic relationships among Australian and American isolates of bean rust. In the present investigation one class of molecular marker, RAPDs, was selected to compare bean rust collections because of its technical simplicity and the ready ability to observe genetic markers at many loci. The survey of the Australian population was extended to 42 isolates, and compared with five isolates representing the two most diverse American clusters identified previously by isozymic analysis. The results indicated that the common background genotypes in Australian populations appear to differ from those common in North America, and provided information on the underlying genetic relationships among these geographically isolated sets of isolates.

MATERIALS AND METHODS

Fungal isolates and DNA extraction. The 42 Australian isolates of *U. appendiculatus* used in this study were collected from a number of locations in eastern Australia (Table 1): isolates 1 through 12 were collected over the period 1974 to 1977 (1) and stored under liquid nitrogen by the National Cereal Rust Laboratory of the University of Sydney (now located at Cobbitty, NSW), with the remainder collected during 1989 to 1992 (see Results for further information on the Australian isolates). The origins of the five American isolates have been described previously (14,15), and are summarized in Table 1 and the Results. Cultures were stored as dried, frozen urediniospores at –60 to –70°C (14) in Brisbane (Australian isolates) or St. Paul, Minnesota (American isolates). Owing to restrictions imposed by quarantine regulations, we limited the study to an analysis of background genotype using RAPD markers, and did not attempt to compare virulence phenotypes under uniform conditions at one location.

All Australian isolates were multiplied from a single pustule and further increased on the susceptible cultivar Purple King as described previously (6). Urediniospores were collected, stored, and germinated as described previously for the Australian (4) and American (14) isolates. DNA was extracted from the germinated urediniospores using a rapid small-scale method (27) for the Australian isolates. DNA from the American isolates, extracted from germinated urediniospores by adaptation of the method of Dellaporta et al. (7), was dried in vacuo and mailed to Australia for RAPD analysis. DNA concentrations were estimated with a fluorimeter as described by the manufacturer (Hoefer Scientific Instruments, San Francisco, Calif.).

RAPD analysis. DNA from each isolate was amplified by polymerase chain reaction (PCR) using a Perkin-Elmer Cetus Thermal

cycler. Ten arbitrary, decanucleotide primers were used, one of which (K-10 5′ATTGCGTCCA3′) was synthesized by the Queensland Institute for Medical Research. The other nine were chosen from kits supplied by Operon Technologies: A-01 5′CAGGCCCTTC3′; A-02 5′TGCCGAGCTG3′; A-03 5′AGTCAGCCAC3′; A-05 5′AGGGGTCTTG3′; H-05 5′ATGCGTCCCC3′; H-07 5′CTGCATCGTG3′; I-01 5′ACCTGGACAC3′; L-01 5′GGCATGACCT3′; T-01 5′GGGCCACTCA3′. The first

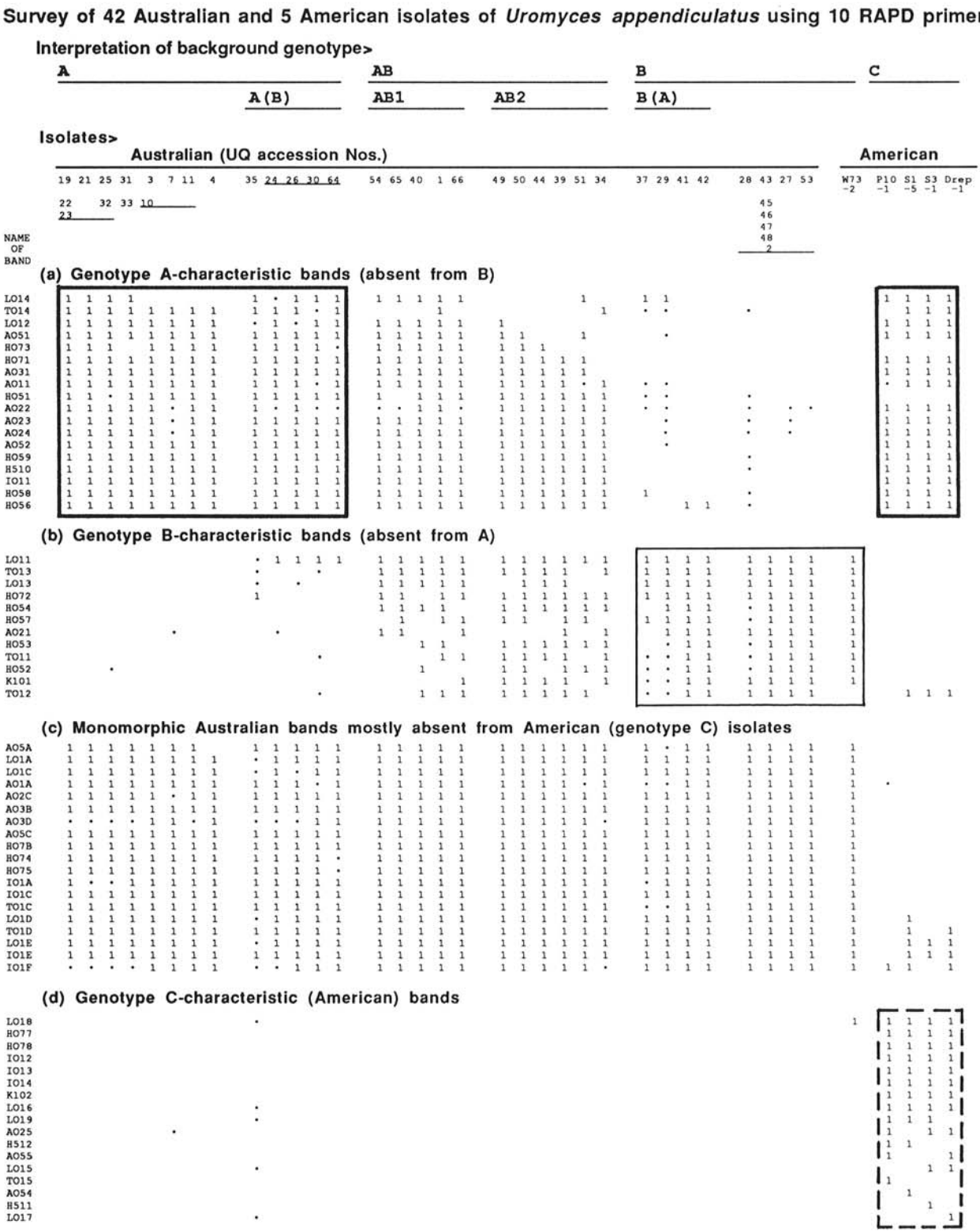

Fig. 1. "Graphic display" of all polymorphic DNA bands detected during a survey of 42 Australian and five American isolates of *Uromyces appendiculatus* var. *appendiculatus* using 10 random amplified polymorphic DNA primers. Bands have been collated and arranged graphically to indicate polymorphisms common to groups identified by cluster analysis (Fig. 3). Monomorphic bands common to all 47 isolates have been omitted. Bands have been named according to the Operon primer and are numbered from lowest to highest molecular weight on the stained gel. The presence of a band in each isolate is recorded as "1," and the absence of a band is indicated by a blank space; missing data are recorded as "•". Monomorphic isolates are listed under each other; a line under groups of adjacent isolates indicates that except for "missing data" they were monomorphic for all bands scored. Blocks of bands characteristic of particular genotypes are enclosed in boxes: heavy boxes = bands characteristic of genotype A (also given by genotype C); light box = bands characteristic of genotype B; dashed box = bands characteristic of genotype C.

six primers listed above were used in the study of Braithwaite et al. (3) and the other four were used with bean rust for the first time in this investigation. The 11 isolates surveyed previously with the first six primers (3) were re-assayed independently with all primers for the present investigation.

PCRs were performed in 0.025 ml containing 67 mM Tris-HCl pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.2 mg per milliliter gelatin, 4 mM $MgCl_2$, 0.2 mM each dATP, dCTP, dGTP, dTTP, 100 to 200 nM primer, and 5 to 30 ng template DNA. Trial PCR reactions were performed both to obtain the optimal DNA dilution for each sample, and to provide replicate reactions. The reactions were heated at 94°C for 4 min before adding 1.6U "Tth plus" DNA polymerase (Biotech International). This was then overlaid with a drop of liquid paraffin. The program cycle was 1 min at 94°C, 1 min at 37°C, 2 min at 72°C, for 39 cycles, followed by 72°C for 7 min. The amplified products were then separated on a 1.5% agarose gel using Tris-borate buffer and detected by staining with ethidium bromide.

Data analysis. All RAPD bands were scored independently and without reference to our previous study (3). For each primer, all DNA fragments that gave a clear band on ethidium bromide–stained gels were assigned the same name (Fig. 1), including bands of identical electrophoretic mobility that differed in intensity among isolates. Some isolates produced faint bands that were not seen clearly with other isolates or in replicate amplifications; such bands were not scored. Bands were recorded as present or absent for each isolate, and data pooled from the 10 RAPD primers were converted to an index of dissimilarity (1-*F*) for each pairwise comparison of isolates. *F* (similarity) values were calculated using the formula $F = 2m_{xy}/[m_x + m_y]$, where m_{xy} = the number of shared bands, and m_x and m_y are the number of bands in each isolate (22). The SAS computer program (SAS Institute, Cary, N.C.) was used for cluster analysis of the (1-*F*) values (McQuitty procedure of SAS, based on the Unweighted Pair Group method with Arithmetic Mean, UPGMA), and the Proc Tree procedure of SAS was used to generate a phenogram. On some occasions, poor PCR amplification of DNA from some isolates was observed with certain primers. Although we repeated many such reactions to obtain a secure result, clear scorable data were not obtained for some primer/isolate combinations because of poor amplification. In these instances, when it was not possible to score with confidence a band that was present in other isolates, we recorded "missing data" to indicate incomplete results. When calculating *F* values in each pairwise comparison of isolates, bands classed as "missing data" from one isolate were disregarded by the computer program even if present in the other isolate.

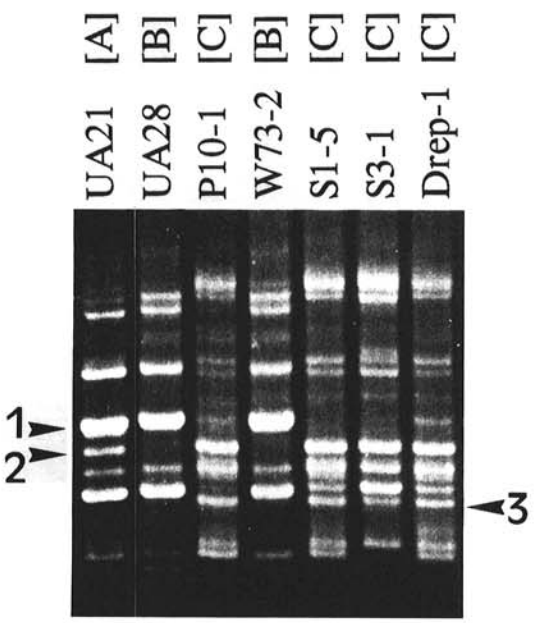

Fig. 2. Agarose gel electrophoresis (ethidium bromide stain) showing random amplified polymorphic DNA bands of five American and two representative Australian isolates (UA21, UA28) of *Uromyces appendiculatus* var. *appendiculatus* (Table 1) using the decanucleotide primer I-01. DNA fragments ranged in size from about 2,000 base pairs (top of photograph) to 500 base pairs (bottom). Background genotype of each isolate is indicated after the accession code. Some examples of the many polymorphic bands on this gel are indicated: (1) bands characteristic of genotypes A and B only, (2) genotypes A and C only, and (3) genotype C only.

RESULTS

The survey. To provide a reasonable chance of detecting diverse genotypes, the 42 Australian isolates surveyed during this investigation were chosen from a variety of geographically diverse locations over eastern Australia collected during the periods 1974 to 1977 and 1989 to 1992 (Table 1). Two sets of isolates, 13 through 19 and 27 through 39, were each collected from a "bean rust nursery" representing an international set of bean cultivars (24) grown in the same field (Table 1). The survey included an independent re-analysis of 21 of the 22 isolates examined previously (3), and included 11 of the subset of 12 isolates that had been characterized using both RFLPs (from 10 cDNA probes) and RAPDs (10 primers). The five American isolates, collected from the northern U.S. and the Dominican Republic over the period 1973 to 1977 (Table 1), were previously characterized for isozyme phenotype as part of a survey that included 23 naturally occurring isolates from diverse locations in North and Central America (14). These isolates were chosen to represent the two most diverse isozyme clusters identified previously (14): isolate W73-2 represented isozyme "cluster 1" (14), whereas isolates P10-1, S1-5, S3-1, and Drep-1 represented divergent subclusters within "cluster 3." DNA from each of the isolates listed in Table 1 was amplified using 10 decanucleotide RAPD primers. An example of banding patterns given by DNA amplification products after agarose gel electrophoresis is presented in Figure 2.

Cluster analysis of RAPDs. Dissimilarity values (1-*F*) calculated from monomorphic and polymorphic DNA bands were used to generate a phenogram by means of UPGMA cluster analysis (Fig. 3). All Australian isolates and one of the American isolates (W73-2) formed a large, dispersed cluster (components labeled "A," "AB," and "B") that was genetically very divergent from another cluster, "C," composed of the other four American isolates. Isolates within the large dispersed cluster were designated A, B, and AB background genotypes, based on our previous survey of a subset of Australian isolates (3) and criteria described later in relation to Figure 1. More heterogeneity was observed within each cluster of A and B genotypes than in our previous study (3). However, consistent with our previous study (3), the cluster of AB genotypes, representing putative hybrids and recombinants between the A and B genotypes, was itself quite heterogeneous.

Graphic display of polymorphic DNA bands. To compare the distribution of particular RAPD bands among the 47 isolates of bean rust, we have omitted all monomorphic bands and arranged the polymorphic bands in a graphic display (Fig. 1). Isolates were first grouped across the top of Figure 1 in the order obtained by UPGMA cluster analysis (Fig. 3), followed by manual rearrangement of isolates when it was judged this assisted the interpretation of data. Bands exhibited by most isolates of genotype A but mostly absent from genotype B were collated and placed at the top of the figure, and termed "Genotype A-characteristic bands" (Fig. 1A). Directly underneath Figure 1A we collated "Genotype B-characteristic bands" that were typical of genotype B but mostly absent from A (Fig. 1B), followed by bands monomorphic to all the Australian isolates and W73-2 but absent from

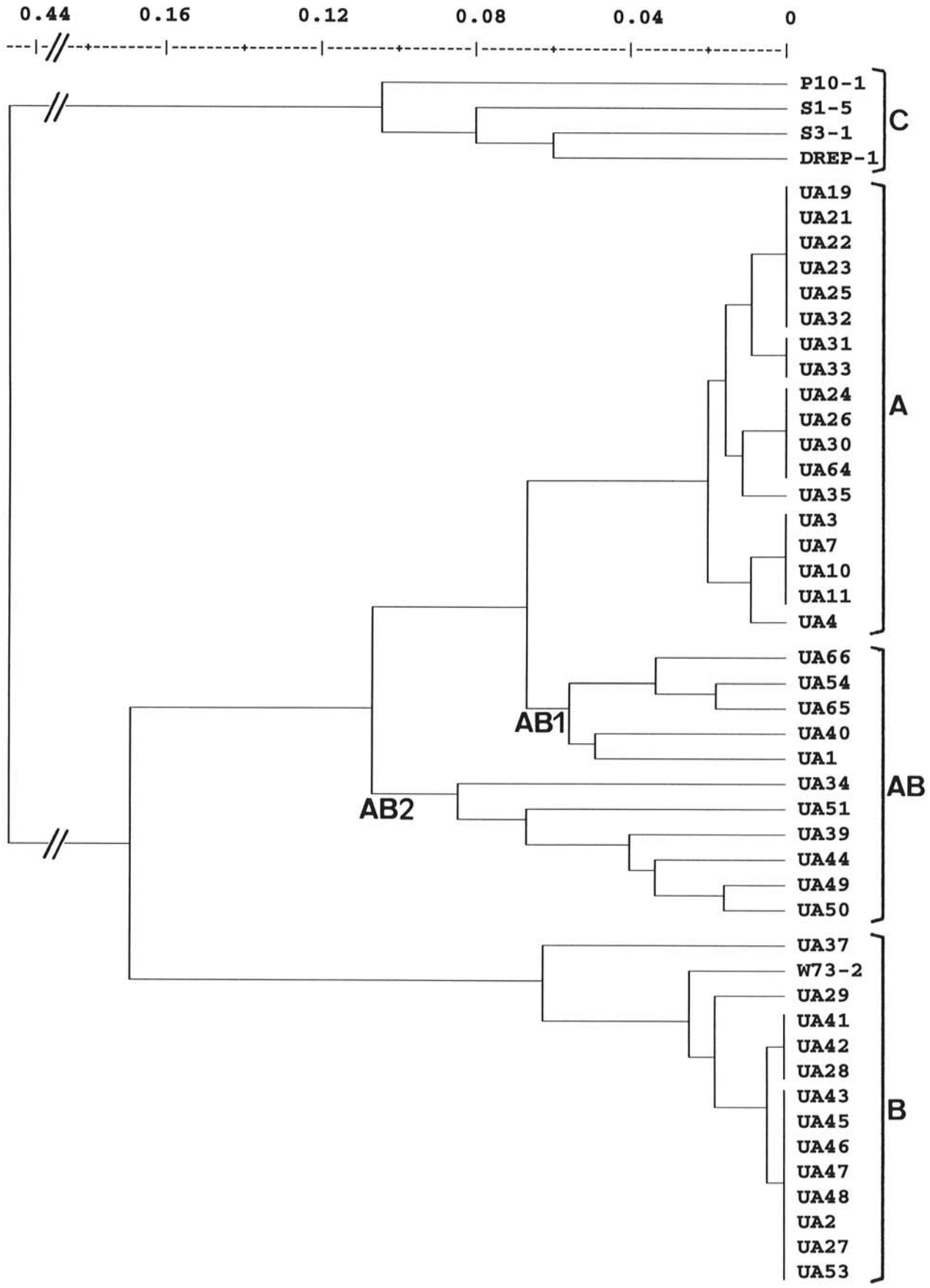

Fig. 3. Phenogram of 42 Australian and five American isolates of *Uromyces appendiculatus* var. *appendiculatus* (Table 1), presented as dissimilarity values (1-*F*) (22) using data pooled from 10 random amplified polymorphic DNA primers. The UPGMA method was used for cluster analysis. Codes on phenograms indicate isolates as listed in Table 1, and the letters A, AB, B, and C indicate clusters of isolates with similar background genotypes (cf. Fig. 1).

all or some of the genotype C American isolates (Fig. 1C). Finally, we collated bands absent from all the Australian isolates and W73-2, but exhibited by some or all of the genotype C American isolates (Fig. 1D).

The graphic display (Fig. 1) shows that 13 of the 18 isolates labeled as genotype A in the phenogram (Fig. 3) exhibited none of the genotype B-characteristic bands. The other five isolates of genotype A, identified as "A(B)" in Figure 1, each exhibited one of the genotype B-characteristic bands. Nine of the 13 isolates labeled as genotype B in the phenogram (Fig. 3) exhibited none of the genotype A-characteristic bands (Fig. 1). The other four isolates of genotype B, identified as "B(A)" in Figure 1, each exhibited one or two of the genotype A-characteristic bands.

Each of the 11 isolates in the group labeled AB in the phenogram (Fig. 3) reproduced 50% or more of both the A- and B-characteristic bands (Fig. 1). These isolates were designated the genotype AB as proposed previously (3), to signify that they appeared to be derived from genotypes A and B by hybridization or recombination. Each of these AB isolates gave its own distinctive banding pattern composed of a different combination of A- and B-characteristic bands (Fig. 1). Although the UPGMA method split these 11 isolates among two subclusters labeled AB1 and AB2 (Fig. 3), the graphic display (Fig. 1) showed no clear distinction between the subclusters. However, the isolates in AB1 generally exhibited relatively more of the genotype A-characteristic bands and relatively fewer of the genotype B-characteristic bands than did isolates in AB2. As found previously (3), no member of the AB group of isolates exhibited any unique polymorphic bands that were absent from the A, B, and C groups (Fig. 1).

Phenetic analysis (Fig. 3) showed a relatively large, 45%, average dissimilarity between the cluster representing genotype C and the cluster representing genotypes A, AB, and B. However, the graphic display of polymorphic bands (Fig. 1) also revealed a strong similarity between genotypes C and A that was obscured by the phenetic analysis. Figure 1A shows that 16 of the 18 bands characteristic of genotype A were also produced by isolates representing genotype C (Fig. 1A). In contrast, 11 of the 12 bands characteristic of genotype B were absent from genotype C (Fig. 1B). The four isolates representing genotype C differed among each other for five of the 19 bands common to all Australian isolates (Fig. 1C), and 9 of the 17 bands absent from all Australian isolates (Fig. 1D).

Reproducibility and "missing data." Good agreement was obtained in the present study with 11 isolates that were independently characterized in a previous study (3) using both RAPDs and RFLPs. Ten of these 11 isolates were classified into the same genotype groups (A, AB, and B) by both studies. The other isolate, UA24, also fell into the same basic genotype (genotype A) in both studies, but in the present study it also produced a single band characteristic of genotype B and fell within the A(B) subgroup of genotype A.

Experience showed that the RAPD technique was prone to give poor amplification reactions on some occasions with some DNA samples. For example, DNA from isolate UA28 gave good amplification when first extracted (Fig. 2), but amplification quality diminished after storage of the DNA (Fig. 1). Since this investigation involved screening a large number of isolates of this biotrophic fungus with many decanucleotide primers, it was not feasible to repeat all inconclusive amplification reactions. When a DNA sample gave generally poor amplification with a particular primer, absent bands that were produced by other isolates tested at the same time were recorded as "missing data" (Fig. 1). Apart from isolates UA37, UA29, and UA28, poor amplification was only observed occasionally, and would have little (if any) impact on the groupings of isolates presented in Figure 1. Although fewer than 8 primers gave reliable data for isolates UA37, UA29, and UA28, these three isolates were clearly excluded from genotype A, and exhibited a predominantly B genotype both in this study and in previous analyses (3). On no occasion were strong (scorable) bands unique to any particular Australian isolate observed, and when poor amplification occurred it resulted in the loss of some of the less intense bands without the generation of spurious new strong bands. Bands of low intensity that were unique to any particular isolate were not scored. Poor reproducibility with RAPDs is a common experience of many workers (2,13), and can cast doubt on the reliability of genetic analyses based solely on RAPDs.

Although some bands scored in the present investigation may be considered doubtful for the reasons outlined above, we offer two arguments for the overall reliability of our data. First and most important, this study follows on from a previous (and independent) investigation of a subset of the same Australian isolates. in which RAPDs and cDNA-generated RFLPs led to identical conclusions in the classification of individual isolates into A, AB, and B genotypes (3). Second, many isolates collected from the Australian population appeared to be clonal or near-clonal (cf. the A and B genotypes in Figures 1 and 3) and acted as internal controls for reproducibility during our analyses. The lack of unique scorable bands for any of the Australian isolates confirmed that few (if any) bands were generated spuriously by chance. This clonality was evident in the many separate experiments that were required to screen all isolates in the survey with each primer. Similarly, although the four American isolates subsequently classified as genotype C were known (and chosen) to be genetically different from each other on the basis of isozymic evidence (cf. 14), they shared most bands in common, and hence acted as controls for each other when scoring common (and dissimilar) bands compared with the Australian isolates.

DISCUSSION

During this investigation we compared the background genotypes of representative isolates of *U. appendiculatus* var. *appendiculatus* collected from cultivated common bean in Australia, North America, and the Dominican Republic. The RAPD genetic markers used to achieve this goal are believed to provide a multilocus comparison by "scanning" the DNA of isolates at loci arbitrarily selected across the genome (3,26). The extended survey of isolates using 10 RAPD primers reported herein, divided the Australian isolates into the same background genotype groups (described below) as a previous study using both RAPDs (10 primers) and RFLPs (10 cDNA probes) (3). Polymorphisms detected in this investigation were subjected both to phenetic analysis (UPGMA method), and a supplementary method of analysis we term graphic display.

Phenetic analysis of RAPD data pooled from 10 PCR primers split the 47 isolates tested into two divergent clusters (Fig. 3). One cluster, composed of subclusters designated A, AB, and B, included all 42 Australian isolates and one American isolate. The second cluster, designated C, included the remaining four American isolates. Background genotypes represented by subclusters A, B, and AB were demonstrated previously among the subset of 12 Australian isolates using both RAPDs and RFLPs (3), and no further diverse Australian genotypes were found during the present investigation. Thus, isolates representing genotype C appear to be either absent or uncommon in Australia.

Genetic relationships among the genotypes identified by phenetic analysis were clarified by graphic display of the polymorphic RAPD bands (Fig. 1). Graphic display enabled us to identify blocks of RAPD bands common to different clusters of isolates, regardless of their average phenetic dissimilarity. Consistent with our previous study of 12 isolates (3), analysis of the expanded survey of 42 Australian isolates by graphic display demonstrated that AB isolates lacked any unique polymorphic bands of their own, and shared various proportions of the polymorphic

bands that distinguished isolates of genotype B from genotype A. Unique RAPD bands can be considered to represent genetic markers that are generated (or lost) by mutation during the evolution of diverse genomes from a common ancestor (3). If so, the expanded survey supports our previous hypothesis (3) that isolates with an AB genotype have most probably arisen from recent hybridization between parental A and B genotypes. Further aspects of the RAPD data that are consistent with hybridization and recombination within the Australian and American populations will be considered below.

Of the 42 Australian isolates surveyed, 18 were classified as genotype A and 13 as genotype B, based on blocks of characteristic bands that distinguished these genotypic groups from each other (Fig. 1). Some isolates within each of these two groups also exhibited one or two bands characteristic of the other group. These isolates are designated A(B) in the genotype A group, and B(A) in the genotype B group (Fig. 1), to indicate the possibility that small genomic elements from one genotype may have introgressed into the genetic background of the other, e.g., by backcrossing of an AB hybrid to a parental A or B genotype. In this regard, an extensive RAPD screen of near-monomorphic isolates of *Colletotrichum gloeosporioides* revealed only two polymorphic RAPD bands, each of which was shown to represent a minor chromosomal element that had introgressed into one strain of this fungus (16,17). The data analysis presented in Figure 1 also drew attention to the variable proportions of A- and B-characteristic bands that distinguished the 11 members of the AB genotype group from each other. Thus, the AB group appears to represent a continuum of isolates that would be expected to result from recombination among an outbreeding population of AB hybrids and their parents. A large proportion of Australian isolates appeared to retain "parental" A or B genotypes (Fig. 1), suggesting that there may be limits to the frequency of hybridization among some members of the current population. In this regard, overwintering via telia is not essential for survival of the fungus in the relatively mild Australian climates from which collections were made. It is also possible that the parental A and B genotypes show climatic or cultivar preferences that assist their maintenance within Australia.

The five American isolates used in this investigation were selected to represent the two divergent genetic groups ("cluster 1" and "cluster 3") detected previously in an isozymic analysis of 23 geographically diverse isolates collected from North and Central America (14). The isolate representing the less common isozyme group (cluster 1) in North/Central America, W73-2, was found to fit with the Australian isolates of genotype B in the present investigation using RAPD markers. The remaining four isolates, representing the prevalent isozymic group (cluster 3) in North/Central America, formed a distinct cluster classified as genotype C in the present investigation using RAPD markers. These four isolates showed appreciable diversity among each other both in the isozymic study (14) and in the present investigation (Fig. 3). Thus, RAPD and isozymic markers showed good congruence in revealing the genetic diversity among isolates of *Uromyces appendiculatus* collected from North and Central America. Phenetic analysis of the RAPD data (Fig. 3) indicated that the genotype C group of isolates was about 45% dissimilar (1-*F*), on average, from the other isolates in the survey representing the A, B, and AB genotypes. This compares with an average dissimilarity of about 17% between genotypes A and B, up to 11% between genotypes A and AB, and 6 to 11% among isolates within genotype C (Fig. 3).

The phenetic data summarized above suggested that genotypes A and B, and their putative AB hybrids, represent a group of isolates that are very divergent genetically from the genotype C group of isolates. It was somewhat unexpected, therefore, when graphic display of individual polymorphic bands revealed a block of bands common to genotypes A and C (Fig. 1A) but absent from B (Fig. 1B). That is, the four isolates representing genotype C possessed almost all of the genotype A-characteristic RAPD bands but very few of the genotype B-characteristic bands. This finding illustrates an inherent limitation of many commonly used methods of cluster analysis such as UPGMA, which, by averaging all differences in RAPD bands among clusters of isolates, can obscure important subsets of RAPD identities among other subgroups, and emphasizes the importance of identifying such relationships by supplementary methods of data analysis such as that presented in Figure 1. An analogous approach was developed by Forster et al. (10) to analyze the distribution of groups of overlapping polymorphic RFLP bands among 48 isolates of *Phytophthora sojae*, a homothallic oomycete pathogenic on soybean. Because some races exhibited polymorphic bands derived entirely from two other races, it was suggested that many current races of *P. sojae* in North America appear to have been derived by recent, rare outcrossing among four "progenitor" genotypes, rather than by successive mutations from the progenitors (or common ancestors).

It is important to consider whether the RAPD data obtained during the present investigation are sufficiently reliable to reveal recombination events among genotypes represented in natural populations of *U. appendiculatus*. Although further research is necessary to establish the veracity and genetic status of any particular polymorphic RAPD band, we present four arguments in support of our analyses, based on multiple polymorphic bands obtained independently. First, as noted previously, an independent survey of a subset of the same Australian isolates used in the present investigation gave very similar patterns of polymorphisms with both 10 RAPD primers and 10 low-copy RFLP probes; the data from each class of genetic marker supported the hypothesis that AB genotypes were derived by hybridization/recombination between "parental" A and B genotypes (3). Second, in the present investigation we scored a total of 18 RAPD bands characteristic of genotype A but absent from B (Fig. 1A); 16 of these bands, produced by nine of the 10 primers tested, were also present in genotype C. Conversely, of the 12 RAPD bands (produced by 6 of the 10 primers tested) that were characteristic of genotype B but absent from genotype A, only one band was also produced by genotype C (Fig. 1B). The consistency of these data, obtained independently with most of the primers tested, supports the view that genotype C shares authentic (i.e., not fortuitous) genetic markers in common with A but absent from B. Third, to evaluate the frequency at which nonhomologous RAPD amplicons fortuitously share the same electrophoretic mobility ("false homologs"), our laboratory compared RAPD homologies among the fungi *U. appendiculatus*, *Phytophthora sojae*, *Colletotrichum gloeosporioides*, and *Metarhizium anisopliae* as controls during a survey of genetic diversity in *M. anisopliae* (8). Using 10 decanucleotide primers and band-scoring procedures similar to those of the present investigation, we found (1-*F*) values ranging between 0.9 and 1.0 among isolates representing each of these different species. Assuming that such divergent taxa should have virtually no homologous bands in common, this range of *F* values should represent the frequency of false homologs; actual *F* values for *U. appendiculatus* compared with the other fungi ranged from 2 to 7% (M. Fegan, *unpublished data*). Taking 10% as an upper limit, we would expect that the 18 bands characteristic of genotype A and absent from B (Fig. 1A) might share up to two false homologs with genotype C, yet 16 homologous bands were observed, suggesting that at least most were true homologs. Conversely, the 12 bands characteristic of genotype B but absent from A (Fig. 1B) might be expected to share about one false homolog with genotype C; only one homologous band was observed, suggesting that genotypes A and C really do differ for this block of genetic markers. Fourth, a total of 30 bands distinguished genotype A from genotype B (18 bands produced by A, 12 by B), 17 of which were also produced by genotype C. If all 17 matches

were fortuitous, then the probability of them being distributed as 16 matching A bands and one matching a B band (or 16 B and 1 A, or 17 A, or 17 B), would be calculated as 0.02%, using the binomial distribution. The second, third, and fourth arguments presented above would be invalid if there were any bias in scoring but, as noted previously, the bands were scored "blind" without any notion that a special homology might exist between genotypes A and C. We therefore suggest that the 16 bands in common between genotypes A and C, as presented in Figure 1A, are best interpreted as representing a block of homologous genetic markers. In future research, selected RAPD bands that indicate homology between genotypes A and C can be verified as true homologs, for example by gel blots and hybridization, or cloning and sequencing to obtain sequence-tagged sites, prior to use for the analysis of expanded sets of isolates of diverse origin.

To account for the distribution of RAPD markers among isolates of *U. appendiculatus* revealed by Figure 1, a possible scheme for the origin of background genotypes is proposed in Figure 4. This scheme postulates the evolutionary development of genetically divergent "B" and "C" lines from a common ancestor, perhaps during coevolution as pathogens of geographically isolated populations of wild beans (discussed below). Recent outbreeding between current B and C genotypes could produce a BC recombinant that lost some of the B-characteristic RAPD markers (Fig. 1B), but gained some C-characteristic markers (the "A-characteristic" RAPD bands in Figure 1A). For example, backcrossing a BC hybrid to a B parent would yield a recombinant genotype that is genetically closer to B than C, as exemplified by genotype A. Alternatively, part of the genome of a C genotype or its relatives could move into a predominantly B background, e.g., via whole chromosomal exchange during sexual or parasexual recombination. This latter possibility is consistent with genetic analysis of a synthetic "semisexual" BC hybrid designated PW3-1, produced by fertilizing pycnia of isolate P10-1 (shown to have a C genotype in the present study) with urediniospores of W73-2 (genotype B) (19). Selfing analysis of PW3-1 (18) demonstrated biased segregation toward the sexual "C" parent and nonrandom assortment of chromosomes. An opposite F2 segregation bias, perhaps from BC hybrids generated from relatives of the current North American B and C genotypes infecting wild bean, could lead to the formation of an A-like genotype. In support of the above hypotheses, a Maryland isolate, R32-l, which exhibited an isozyme phenotype almost identical to that of the synthetic BC hybrid PW3-1, appears to represent a naturally occurring BC hybrid (14).

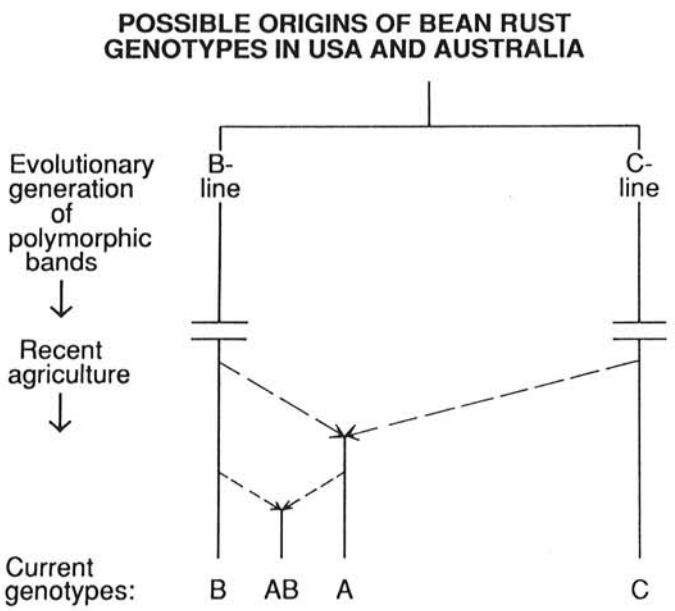

Fig. 4. Scheme showing possible evolutionary relationships leading to the formation of the current background genotypes of *Uromyces appendiculatus* var. *appendiculatus* pathogenic on cultivated bean in the U.S. and Australia. It is postulated that genotype A was formed by the introgression of genes from C into B, and that B and A (but not C) entered Australia where they have continued to exchange genes. The scheme was deduced from the distribution of polymorphic random amplified polymorphic DNA bands presented in Figure 1.

The many genetic differences between genotypes B and C detected by RAPDs may be interpreted as a consequence of the coevolution of bean rust with its host. Several lines of evidence indicate that *Phaseolus vulgaris* was domesticated a number of times from its wild progenitor species at centers of origin ranging from Mexico (Mesoamerican center) to Peru/Argentina (Andean center) (11,23). It has been suggested that some bean pathogens may have diverged, to specialize on beans representing divergent germ plasms that were domesticated independently (11). Among North American cultivars, green (French or snap) beans and red kidney beans are derived from the Andean center, while the better-known dry types (navy, pinto, and great northern beans) are Mesoamerican. Groth and Shrum (12) found that American collections of *U. appendiculatus* that we now believe to belong to genotype C (by RAPD comparisons with isolates used in the present study, J. V. Groth and J. P. Martinez, *unpublished*) are strongly adapted to dry beans, while the collection with a representative in this study, W73 from genotype B, is strongly adapted to French and red kidney beans. It is therefore possible that genotypes B and C of bean rust represent components of the subpopulations of *U. appendiculatus* that were originally adapted to the Andean and Mesoamerican centers of domestication of wild bean, respectively. Maintenance of large genetic differences between genotypes B and C would require that genetic recombination be quite restricted between these two genotype groups, and it is possible that genotypes B and C each evolved in genetic isolation on geographically separated populations of wild bean. This hypothesis is consistent with the high proportion of polymorphic RAPD markers that distinguish genotypes B and C (Fig. 1).

The apparent absence of genotype C from Australia is suggestive of a bottleneck in the introduction of *U. appendiculatus* from its center(s) of origin in the Americas. Analysis of cryogenically stored material demonstrated that genotypes A and AB at least, and possibly B also, were present in Australia before 1974 (3). The A, AB, and B genotypes so far identified in Australia suggest that *U. appendiculatus* was introduced either as an outbreeding A+B population, or as separate A and B introductions that subsequently outbred with each other. A source in South America would allow entry to Australia of airborne spores via globe-circling prevailing winds in the Southern hemisphere, as proposed for the entry of new genotypes of the wheat stem rust fungus to Australia from Africa (5).

Despite such inherent limitations of RAPDs as dominance (21,26) and poor amplification on some occasions, the ready access to large numbers of markers at many loci provided by RAPDs has nonetheless allowed us to formulate reasonable working hypotheses on genetic relationships among selected isolates within the Australian and North American populations. Independent comparison of further random DNA polymorphisms, including co-dominant RFLP markers, sampled across the genomes of members of the A, AB, B, and C genotypes in relation to geographic distribution in the Americas and to pathogenicity tests for Andean/Mesoamerican germ plasm preference, would provide one means of testing these hypotheses. It is probable that the recent relatives of these genotypes may still exist, perhaps as pathogens of wild relatives of *Phaseolus* distributed along its center of origin. Multilocus DNA analyses of such "wild" isolates of *U. appendiculatus* would provide an additional means of deducing the origin and spread of this pathogen worldwide.

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